



# AqualImprove

## 3<sup>rd</sup> AqualImprove - Aquaculture Research Workshop

Book of abstracts

**Editors:** Helena Peres and Aires Oliva Teles

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NUTRIMU – Nutrition and Immunobiology Research Group  
CIIMAR - Interdisciplinary Centre of Marine and Environmental Research  
Terminal de Cruzeiros do Porto de Leixões  
Avenida General Norton de Matos, S/N  
4450-208 Matosinhos | Portugal





## **3<sup>rd</sup> Aqualmprove - Aquaculture Research Workshop**

### **Workshop Coordinators:**

Helena Peres and Aires Oliva Teles

### **Workshop Scientific Committee:**

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## 3<sup>rd</sup> Aqualmprove - Aquaculture Research Workshop

The **AQUAIMPROVE Workshop** aims to promote the presentation and debate of recent research advances in aquaculture-related topics in Portugal and is directed to students, researchers, academic community, and stakeholders.

It follows **AQUAIMPROVE Workshops** held in 2015 and 2017, which largely surpassed 100 participants. The perception of the interest of this initiative encouraged us at NUTRIMU-CIIMAR to promote it as a regular meeting point for the scientific community and stakeholders to discuss issues related to aquaculture.

Of course, the success of these workshops is directly related to the willingness of participants to attend it, but they would not be possible to organize without the pro bono dedication of the local organization committee, composed by the NUTRIMU community. To all of them, our sincere thanks.

The editors

## Scientific program

9:00-10:00 Registration and Poster set-up

10:00 Opening Section

**10:00 Plenary Talk–Progress and difficulties in developing high performance microdiets for fish larvae (Luís Conceição, S. Engrola, W. Pinto, P. Pousão-Ferreira, M. Yúfera)**

**10:20 Coffee break**

### ALISSA Section

*Chair* –Dr. Luís Conceição

10:50 Methionine and tryptophan dietary supplementation induce opposite effects on the European seabass (*Dicentrarchus labrax*) immune response and disease resistance  
M. Machado, R. Azeredo, F. Fontinja, A. Domingues, S. Fernandez-Boo, L.E.C. Conceição, J. Dias, N.M.S. dos Santos, B. Costas

11:05 Short-term feeding with arginine and citrulline improves the gilthead seabream (*Sparus aurata*) immune status  
L. Ramos-Pinto, C. Silva, R. Azeredo, L.E.C. Conceição, J. Dias, T.S. Silva, D. Montero, B. Costas

11:20 Effects of methionine and threonine on oxidative stress and energy allocation in rainbow trout under increased temperatures  
A.C.M. Rodrigues, S.F.S. Pires, A.P.L. Costa, L.E.C. Conceição, M. Castro-Cunha, J. Dias, J. Calheiros, C. Gravato, A.M.V.M. Soares, R.J.M. Rocha

11:35 Effects of amino acid supplementation on the performance of *Sparus aurata* using metabolic fingerprinting proteomics and western blot techniques  
A.P. Farinha, D. Schrama, M. Cerqueira, R. Colen, S. Engrola, T. Silva, P.M.L. Rodrigues

11:50 Quantitative prediction of the effects of dietary factors on fish growth and disease susceptibility using molecular indicators of nutritional and immune status  
T.S. Silva, L. Ramos-Pinto, M. Machado, J.A. Martos-Sitcha, R. Azeredo, J. Caldach-Giner, J. Pérez-Sánchez, S. Engrola, J.M.O. Fernandes, N. dos Santos, B. Costas, L.E.C. Conceição

12:05 Prediction of European seabass growth using the FEEDNETICS metabolic model  
T.S. Silva, F. Soares, R. Colen, D. Schrama, S. Engrola, L.E.C. Conceição

**12:20 Lunch**

**14:00 Plenary Talk: SEAentia – leading technology towards sustainable aquaculture (SEAentia)**

*Chair*–Dr. Benjamin Costas

14:20 Life cycle assessment of diets for gilthead seabream (*Sparus aurata*) with different protein/carbohydrate ratios and fishmeal or plant feedstuffs as major protein sources  
C. Basto-Silva, I. Guerreiro, A. Oliva-Teles, B. Neto

- 14:35 Improving perception and acceptability of aquaculture through experimental science activities for school teachers and their students  
M. Correia, L. Guimarães e M. Almeida
- 14:50 Microbial community in a marine recirculating aquaculture system  
D. Almeida, C. Magalhães, E. Silva, D. Rosado, I. Blanquet, A.P. Mucha
- 15:05 New Molecular tools for labelling and monitoring *Bacillus subtilis* probiotics in vivo  
A.S. Lavrador, A. Oliva-Teles, A. Couto, C.R. Serra
- 15:20 Comparison of gonads from sea urchin *Paracentrotus lividus* fed a commercial diet and wild individuals  
L.F. Baião, F. Rocha, T. Sá, A. Oliveira, M. Pintado, L.M.P. Valente
- 15:35 Aquaculture of native seaweed species  
T.R. Pereira, S. Brito, M. Gonçalves, I. Azevedo, I.S. Pinto
- 15:50 Parasites of *Paracentrotus lividus* (Echinodermata: Parechinidae) off northwest Portugal, northeast Atlantic waters: a problem to be taken into account in culturing systems?  
F.I. Cavaleiro, J.P. Silva, A. Henzel, D.G. Frade, L.F. Rangel, M. J. Santos

**16:05 Coffee break - Poster session**

*Chair - Professor António Afonso*

- 16:35 Microalgae replacement by *Ulva rigida* in Pacific oysters (*Crassostrea gigas*) diet: effects on reproductive success, fatty acid profile and metabolic responses during broodstock conditioning.  
A. Rato, L. Valido, J. Machado, P. Vaz-Pires, J.F.M. Gonçalves, S. Joaquim, A.M. Matias, N. Bandarra, D. Matias, R. Ozório
- 16:50 Effect of extraction method and solvent system on the phenolic content and antioxidant activity of selected macro- and microalgae extracts  
M. Monteiro, R.A. Santos, A. Couto, C.R. Serra, I. Gouvinhas, A. Barros, A. Oliva-Teles, P. Enes, P. Díaz-Rosales
- 17:05 The effect of nitrogen availability in the antioxidant content of *Chenopodium quinoa* under saltwater hydroponics  
M. Murteira, A. Lillebø, J. Papenbrock
- 17:20 Total replacement of fish oil in diets with a high inclusion of plant proteins: effects in nutritional quality and growth of farmed *Senegalese sole*  
R. Pereira, A. Basto, M. Conde-Sieira, F. Linares, J.L. Rodríguez, G. P. Sieira, J.L. Soengas, L.M.P. Valente
- 17:45 Expression of LC-PUFA metabolism genes in juvenile tambaqui (*Colossoma macroponum*) fed different dietary lipid source and levels  
R.B. Ferraz, A.L.S. Modesto, R. Ozório, A.L. Salaro, O. Monroig, L.F. Castro

Sponsors



# Plenary presentations

# 1

## Progress and difficulties in developing high performance microdiets for fish larvae

Lúis Conceição<sup>a</sup>, Sofia Engrola<sup>b</sup>, Wilson Pinto<sup>a</sup>, Pedro Pousão-Ferreira<sup>c</sup>,  
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### Abstract

Major efforts by academia and industry have targeting high performance microdiets for fish larvae since the 1990's. Progress has been considerable, with good weaning results being currently delivered by several commercial microdiets, for major cultivated species. Over recent years, significant progress on weaning has also been achieved for some candidate species for the expansion of the Aquaculture industry. Still, there is room for substantial improvements in microdiets for marine fish larvae, in particular for the very early stages. Even if we know that fish larvae require diets with high levels of protein, essential fatty acids, and micronutrients, and that these are provided by highly digestible ingredients, the exact nutritional requirements are poorly understood. It is also clear that early nutrition has consequences during the larval, but also later in the juvenile stage, in terms of health status, survival, skeletal deformities and growth performance. Fish species have different nutritional requirements, and high-quality ingredients that work very well in a species, may be not suitable for other species. Very early weaning is becoming a reality in several species, and its long-term effects seem to be very positive. Novel ingredients such as microalgae and organic forms of minerals may bring either positive or negative effects on larval quality. Nutrition is always a matter of balance. Moreover, the success of a given microdiet depends also on the feeding techniques used and needs to meet some specifications in addition to nutritional adequacy to the target species. It must ensure a high ingestion, allow easy digestion, prevent nutrient leaching losses; and present optimal properties in terms of floatability, sinking speed, texture, and dispersion both in tank surface and water column.

Leaching losses may compromise larval quality by nutrient loss, as well by reduction of water quality. Microencapsulation can reduce nutrient leaching, but also microdiet digestibility, so a good balance is required. As knowledge on larval nutrition progresses and newer technologies become available, better performing microdiets for fish are becoming available. This faster growing larvae, with higher survival rates, that lead to better quality juveniles. Live feed replacement will increasingly be a reality in the coming years, with many species being able to start feeding exclusively (or almost) on high quality inert microdiets.

João Rito

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### Abstract

SEAentia is a Portuguese aquaculture startup with the aim of producing healthy and sustainable food.

There is an increasing global demand for seafood justifying one of the UN 2030 goals: “Feeding a growing population”. This objective, combined with the fact that consumers are becoming increasingly aware of environmental protection, animal welfare, food quality, food biosafety and sustainability, motivated us to form this company.

To this end, SEAentia aims to be at the forefront for developing scientific approaches and applying new technologies for sustainable and responsible seafood production.

Through a state-of-the-art recirculating aquaculture system (RAS) that is fully powered by sustainable energy sources, we aim to integrate different trophic level productions.

SEAentia will be the first to produce meagre (*Argyrosomus regius*) to commercial harvest size in an onshore RAS. This system will be fully powered by photovoltaic panels covering the roof.

In embracing the concept of the circular economy, our company will channel the biological side-products of fish feeding into raw materials for cultivating additional high-value produce.

SEAentia’s products will be fully traceable and will be presented to consumers with certification for sustainability, environmental protection and biosecurity.



# Oral presentations

# 1

## Methionine and tryptophan dietary supplementation induce opposite effects on the European seabass (*Dicentrarchus labrax*) immune response and disease resistance

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### Abstract

Methionine is an example of an amino acid (AA) with recognized roles in the immune system, DNA methylation, polyamines biosynthesis, and in processes involved in the control of inflammation. Tryptophan participates in an array of physiological mechanisms of the neuroendocrine-immune network and plays a critical role in macrophages and lymphocytes function. Thus, a potential immunomodulatory role of both AA during infection is envisaged and deserves further attention. In this context, two identical trials were performed to evaluate the effects of methionine or tryptophan supplementation on the European seabass inflammatory response and disease resistance to *Photobacterium damsela* piscicida (*Phdp*) after a 4 weeks feeding period. A control diet (CTRL) was formulated to meet the seabass AA requirements, and four CTRL-based diets were supplemented with DL-Methionine at 0.5% and 1% (MET 0.5 and MET 1, respectively) or L-Tryptophan at 0.35% or 0.39% (TRP 0.35 and TRP 0.39, respectively) of feed weight. Methionine supplementation translated into an enhanced immune response to infection, as observed by improved cellular and humoral defences as well as modulation of key immune-related genes. This enhancement of immunity resulted in increased survival of fish fed methionine-supplemented diets and ultimately suggest a positive effect of methionine administration in a dose-dependent manner. Tryptophan supplementation led to a reduction of peripheral blood lymphocytes, monocytes and peritoneal macrophages numbers and down-regulation of several immune-related genes in response to the *Phdp* insult. As a result, fish fed TRP 0.39 presented the highest mortality whereas fish fed TRP 0.35 and CTRL showed similar disease resistance. Therefore, data point out deleterious immunomodulatory effects of TRP 0.39, under the present experimental setup. This study brings relevant insights about the opposite roles of individual AA on the seabass immune system, and their possible application as nutritional additives for fish health management should be carefully considered.

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### Abstract

Several amino acids (AA) are known to regulate key metabolic pathways that are crucial for immune response. In particular, arginine appears to have important roles regarding immune modulation since it is required for macrophage responses and lymphocyte development. Moreover, citrulline is a precursor of arginine, and it has been reported as an alternative to arginine for improving macrophage function in mammals. The present study aimed to explore the effects of dietary arginine and citrulline supplementation on the gilthead seabream immune status in a short-term feeding basis. Triplicate groups of fish ( $23.1 \pm 0.4\text{g}$ ) were either fed a control diet (CTRL) with a balanced AA profile, or the CTRL diet supplemented with graded levels of arginine or citrulline (0.5% and 1% of feed), ARG1 and CIT1 and ARG2 and CIT2, respectively. After 2 and 4 weeks of feeding, fish were euthanized and samples were collected for haematological procedures, and assessment of humoral immune parameters in plasma as well as the expression of health-related biomarkers (e.g. *tgfb*, *csfr*, *tcr*, *il-10* and *il-34*) in the head-kidney. The bactericidal activity increased in fish fed the highest supplementation level of both ARG and CIT dietary treatments compared to fish fed the CTRL diet after 4 weeks of feeding. A tendency of higher relative numbers of circulating monocytes was also observed in fish fed diets with the highest supplementation level after 2 weeks of feeding, compared to their counterparts fed the lower supplementation level. Peripheral monocyte numbers also correlated positively with nitric oxide levels in plasma, which showed an increasing trend in a dose dependent manner. The colony stimulating factor 1 receptor (*csfr*) tended to be up-regulated at the final sampling point regardless the dietary treatments. These results suggest that dietary supplementation with arginine or its precursor (citrulline) have an immunostimulatory effect after 2 weeks of feeding. More health-related biomarkers are being processed which will enlighten the effects of these functional diets.

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## Abstract

Aquaculture is one of the fastest growing food production industries in the world. However, the sustainable development of this activity is facing challenges from climate change. Sub-optimal feeding regimes, with consequent poor feed conversion, physiological effects, additional feeding costs and environmental impacts are still a major issue for the sustainable development of aquaculture.

This study aimed to evaluate if feed formulations supplemented with 2 levels of essential amino acids (methionine and threonine) affected the physiological responses of rainbow trout (*Oncorhynchus mykiss*) to warming waters, and whether these welfares remain after the supplementation period. For that, rainbow trouts were reared at 15 °C, in RAS, and fed with 1 control and 2 supplemented diets for 2 different periods (1 and 2 weeks). Stress stimulus was initiated after 1 week of feeding until a maximum water temperature of 25 °C. Additionally, triplicates of each diet were maintained at 15 °C. After this period, fish were fed exclusively with control diet for another 2 weeks, to evaluate if the benefits of supplementation still occur. Samplings (liver and muscle tissues) were performed every week. The activity of catalase (CAT), glutathione-S-transferase (GST), total glutathione (TG), lipid peroxidation (LPO), cellular energy allocation (CEA) and heat shock proteins (HSP-70) were measured in liver tissue. In muscle tissue, CEA methodology was applied.

Warming waters increased oxidative stress and impaired cellular metabolism of fish fed with control diet. Supplementation with essential AA proved to be effective in the minimization of physiological alterations due to thermal stress. In sum, energetic costs for cellular homeostasis maintenance were observed and will ultimately impact individuals' fitness and resistance under different environmental stressors, reinforcing the need for seasonal supplementation. Integrative approaches encompassing physiological and biochemical biomarkers contribute to fill the gap of understanding fish nutritional requirements and be able to mitigate the effects of climate changing.

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### **Abstract**

**Introduction:** Aquaculture industry is the fastest growing farmed animal sector worldwide. However, in order to sustain such growth rates, alternative protein ingredients need to be included in fish diets. Plant-based protein ingredients are an alternative for fish diets but may influence growth and stress due to imbalanced amino acids profiles. It is important that the plant-based diets used in the aquaculture feeds maintain or increase the levels of feed intake, feed conversion efficiency, growth rate and survival in fish when compared with fish meal (FM) diets. Evaluation of the functional effect of diet supplementation with three amino acids (AAs), like histidine: involved in biochemical changes that occur during inflammation; threonine: involved in the immune system response, and tryptophan: involved in stress and inflammatory responses was performed using differential proteomics (2D-DIGE).

**Methodology:** Juvenile gilthead seabream (*Sparus aurata*) with an initial wet body weight of 13 g, were fed with three experimental diets, which were formulated based on estimated requirements. Diet 1 had no supplementation of the three amino acids (histidine, threonine and tryptophan), diet 2 had 1.1% (w/w) of supplementation and diet 3 was supplemented with 1.5% (w/w). Fish were fed twice a day, *ad libitum* and maintained in a flow-through system, dissolved oxygen in seawater was maintained above 5 mg L<sup>-1</sup> with a mean temperature of 22.5°C ± 2. 7°C. Sampling was performed after 14 and 92 days, fish were lethally anesthetized with 2-phenoxyethanol, liver was collected and kept at -80°C for further analyses. Proteins were extracted using a DIGE buffer and quantified by the Bradford method. Proteins were separated using 2D-DIGE on 24 cm dry strips with pH 4-7. Gels were analyzed using Same Spots software and statistically different spots (ANOVA, p<0.05) between the diets were excised manually and sequenced by MALDI-TOF/TOF. Validation of proteomic results will be performed using western blot with commercial available antibodies. Liver samples of both sampling times were freeze-dried and mixed with potassium bromide to produce a metabolic fingerprint using FT-IR (Fourier-transform Infrared spectroscopy).

**Results:** Identified proteins from samples of 14 days of trial were related to protein transport - namely transportin-2 and a chaperone- namely disulfide isomerase. These proteins were both upregulated in diet 2 (1.1% supplementation with histidine, threonine and tryptophan). In case of sampling after 92 days a protein related to the lipid metabolism - namely 3-oxo-5-beta-steroid

4-dehydrogenase-like and a tumor-suppressor - namely B-cell lymphoma/leukemia 11B-like isoform were both upregulated in diet 3 (1.5% supplementation with the referred AAs). In case of metabolic fingerprinting no significant differences were observed between diets at each sampling point, but in between samplings a shift was observed in the carbohydrate content showing higher amounts after 92 days. It seems that, throughout the trial, energy reserves increased overtime. These results show that the supplemented AAs might influence the functional state of gilthead seabream.

Conclusions: We conclude that, despite small differences in terms of growth performance, the supplementation with histidine, threonine and tryptophan may have influenced the nutritional state of gilthead seabream.

**Acknowledgements:**

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# 5

## Quantitative prediction of the effects of dietary factors on fish growth and disease susceptibility using molecular indicators of nutritional and immune status

T.S. Silva, L. Ramos-Pinto, M. Machado, J.A. Martos-Sitcha, R. Azeredo, J. Calduch-Giner, J. Pérez-Sánchez, S. Engrola, J.M.O. Fernandes, N. dos Santos, B. Costas, L.E.C. Conceição

### Abstract

An increasingly important aspect of fish nutrition research is to evaluate how formulation changes affect fish nutritional and immune status, given the possibility of downstream effects on disease susceptibility and growth performance. The classical approach to these issues is to perform feeding trials and evaluate how fish nutritional and immune status are nutritionally modulated through the assessment of growth performance, along with the measurement of molecular indicators (optionally before and after a nutritional or immune challenge event). This approach has as shortcomings the fact that mid- and long-term feeding trials involve high costs and fact that interpreting changes in molecular indicators can be challenging, even in the presence of positive and negative reference groups, due to the high sensitivity of molecular markers to all sorts of environmental and contextual factors.

In this presentation, we cover some of the work done throughout project ALISSA where we calibrate simple models to predict a long-term phenotype (e.g. “growth performance after 3 months” or “expected mortality rate after a bacterial challenge”) based on short-term gene expression measurements, using data from the project’s seabream and seabass trials. The underlying idea is to enable the assessment of dietary effects on a short-term basis (i.e. weeks, rather than months), while ensuring that indicators are interpreted within an objective quantitative framework, without the need for positive and negative reference groups.

Though the results so far are encouraging, we’ll also discuss the limitations and pitfalls of this approach within the context of fish nutrition research.

T.S. Silva, F. Soares, R. Colen, D. Schrama, S. Engrola, L.E.C. Conceição

### **Abstract**

Given the strong ongoing trend in fish feed formulation of replacing fish meal with alternative sources of protein, it is increasingly important to be able to predict the impact of such changes on fish growth performance. An advantage of using a metabolic model for fish growth prediction, rather than a bioenergetic model, is that the effect of varying dietary amino acid profiles can be more naturally accounted for.

In this presentation, we compare the results of a European seabass growth trial against predictions using the FEEDNETICS fish metabolic model calibrated for European seabass. For this trial, seabass with an initial average body weight of 10 g were fed with one of nine different diets over the course of 85 days. Of the diets used, four were completely devoid of fish meal and presented different levels of methionine inclusion (tested in triplicate), one diet rich in fish meal (tested in duplicate) and four low-FM diets displaying different level of crude protein and crude fat (tested without replication). At the end of this trial, fish fed with the different experimental diets displayed some level of variation in terms of growth performance, reaching final average body weights between 40 and 55 g.

The results show a strong agreement between measured and predicted growth performance, demonstrating the usefulness of using metabolic models to rank and screen diets according to estimated growth performance, within the context of exploring alternative sustainable sources of protein for aquafeeds.



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### Abstract

Aquaculture is the best alternative to fulfil global fish demand, however aquafeeds still rely heavily on fisheries products. This study assesses and compares the environmental impacts of four experimental diets to gilthead seabream including either fish meal (FM) or plant feedstuffs (PF) as major protein sources and the protein (P)/carbohydrate (CH) ratios of P50/CH10 or P40/CH20.

This study used Life Cycle Assessment (LCA) methodology. The functional unit used in the study was 1 kg of diet. The studied boundaries included aquafeed ingredients production (S1), compound aquafeed production under laboratory conditions (S2), and transportation between S1 and S2 locations. The present study applied the Recipe Endpoint method, hierarchist version (V1.13; Europe recipe H/A). The background data was collected from Ecoinvent database and related literature. For each aquafeed ingredient it was accounted either the agriculture production or fishery activities, processing unit, and transportation between the production and processing locations. Ingredients mixing and processing was done at the Marine Zoology Station (MZS) located at Porto, Portugal. It was also taken into account the road transportation aquafeed ingredients between the commercial company and the laboratory.

Regardless of dietary protein source or P/CH ratio, all formulated diets had the same single score index for environmental impact. In agreement with several studies, S1 was the system with the highest environmental impact. On the other hand, the environmental contribution of aquafeed ingredients transportation until the MZS was almost negligible. Fishery-derived ingredients were the largest contributors to the environmental impact. In the hypothesis of replacing the fish oil (FO) from fisheries by-products by FO from Peruvian anchovy fisheries, the environmental impacts of the diets would be decreased.

Studying the replacement of FO from fisheries by-products by Peruvian anchovy FO allowed to understand the importance of ingredients selection. As limitations of the current study it is important to mention: first, the use of cold pelletisation as manufacturing process and, second, the non-valuation of fish by-products.

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### **Abstract**

The Ocean is an important source of countless resources and services of inestimable value. Its protection is therefore essential and a global priority. Although crucial for its protection, integrated understanding of the influence of the Ocean on mankind and the influence of mankind on the Ocean is still rarely addressed in school curricula or in complementary activities offered to young people. It is therefore necessary to promote a comprehensive discussion about integrated knowledge of the Ocean, increasing Ocean Literacy in our country.

Through the development of experimental activities to be carried out by students in their schools or during visits to CIIMAR we intend to increase Ocean Literacy and address a wide variety of topics related to the Ocean. One of the most seek out themes is related to aquaculture, in particular Integrated Multi Trophic Aquaculture (IMTA). This activity allows students to build a small IMTA system and recognize it as a nature-based solution for the sustainable exploitation of marine resources, by minimizing the impact on the environment and fostering economic valuation through the diversification of products. With this hands-on experiment student will work essential aspects of biodiversity, concepts of trophic webs and eutrophication associated with the discharge of effluents enriched in inorganic nutrients, as well as principles of circular economy, and ultimately improve their perception and acceptability of aquaculture.

Teachers also play an important role on raising awareness on the importance of the Ocean on our lives. With the implementation of teacher's training actions on experimental teaching of marine sciences, we purpose to solve the lack of Ocean related topics addressed in school curricula. By dedicating a module of the training action to aquaculture, we intend to show the aquaculture that is done in Portugal and in Europe, and the latest technological innovations and advances in this area, thus promoting aquaculture positive acceptability and perception. In this work we will also present the results of preliminary questionnaires made to the teachers that attended the training action, to assess their perception of aquaculture before and after the module about aquaculture.

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### Abstract

Recirculating aquaculture systems (RASs) provide opportunities to reduce water usage and to improve waste management and nutrient recycling, making intensive fish production compatible with environmental sustainability. Managing disease outbreaks poses specific challenges in a RAS in which a healthy microbial community contributes to water purification and water quality. As so, the aim of this work is to study the microbial communities from a marine RAS, with special emphasis on the pathogenic *Tenacibaculum mairitimum* (*T. maritimum*) bacteria, in order to understand and manage its infections throughout the production process.

Fish tissue and water samples from the marine RASs system in study were investigated for *T. maritimum* detection and isolation using procedures previously described. *maritimum* was detected in fish tissue samples (eggs and larvae) in the earliest life stages and in the recirculating water system with a nested conventional PCR approach using specific primers. Isolation of *T. maritimum* from water samples was attempted using selective mediums, however only *T. ascidiaceicola* was successfully isolated.

Currently, the detection and quantification of *T. maritimum* by quantitative Real-Time PCR is being optimized using water of different compartments inside the system and fish tissues samples in different life stages for further monitoring of the RAS system. Furthermore, 16S rDNA amplicon-based Next Generation Sequencing (NGS) technology are being tested as an alternative approach for the detection and quantification of *T. maritimum* in different RAS matrices (water/biofilm/different fish stages and organs). Moreover, this procedure will be performed in other to evaluate changes in the microbial community between the different RAS compartments and under environmental alterations.

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### Abstract

In aquaculture, the intensification of fish production often leads to stress, rapid transmission of pathogens and consequently increases the occurrence of diseases. In 2006, the European Union banned the use of antibiotics as growth promoters in animal production, including aquaculture, and probiotics emerged as an alternative to improve fish growth while preventing fish diseases. Probiotic bacteria are used to manipulate the host's gastrointestinal microbiota to increase the ratio of beneficial bacteria, through competition for adhesion sites and nutrients or production of antimicrobials, but the exact mechanisms of action remain not fully understood. To help on this clarification, several studies have tried to determine the dissemination path of probiotics in the gastrointestinal tract of both terrestrial and aquatic animals, but none resulted in real time visualization of the bacteria in the host's digestive tract.

In this work, we designed a molecular tool that allows *in vivo* monitoring of *Bacillus subtilis* probiotics. To do so, a plasmid, pASL1, containing the green fluorescent protein GFP under the control of a constitutively expressed promotor (*Pveg*), was created and successfully integrated into the chromosome of the laboratory *B. subtilis* 168 strain as well as into a probiotic fish isolate of *B. subtilis*. The use of *Pveg* allowed cell-labelling of different morphological stages that *B. subtilis* assumes due to its spore forming nature (vegetative and sporulating cells, adding to free spores), an essential tool to understand how and where *B. subtilis* probiotics persist and disseminate within the gastrointestinal tract, and where and when germination and probiotic establishment might occur.

Growth, sporulation and *in vivo* visualization of the fluorescently-labelled bacterial derivatives (CRS201 and CRS202) were tested and validated, outside and inside *Brachionus plicatilis rotifers*, in a modified culture media with salinity adjusted to the level at which rotifers are cultivated (15ppt). The *in vivo* trial showed that both strains accumulated inside the gastrointestinal tract of *B. plicatilis* within 1 hour after inoculation and were visible inside the rotifer during the course of 5 hours. This observation indicates that recombinant probiotic strains, carrying the molecular tool developed in this work, can be efficiently monitored in the gastrointestinal tract of rotifers, as well as other optically translucent invertebrates and vertebrates. By using rotifers as a vehicle for probiotics administration to fish, this tool will create a visual path of *B. subtilis* probiotic location and dynamics within the gastrointestinal tract of zebrafish, allowing to gain further insights on probiotics mode of action for their use in aquaculture.

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### Abstract

In the Mediterranean Sea, the sea urchin (*Paracentrotus lividus*) gonads have been considered a prized seafood product and a delicacy due to its flavour and texture. The increasing market demand for sea urchin roe has resulted in the depletion of wild stocks becoming an interesting target species for aquaculture production. A practical low-fat diet, with 6% lipids and 30% crude protein (D30), was formulated and fed to three homogeneous groups of 15 individuals (35 g mass; 4.5 cm diameter) harvested in an intertidal zone of North Portugal. Sea urchins were distributed by cages (45 urchins/m<sup>2</sup>) in a saltwater recirculation system (salinity 35‰, 18°C) and fed the experimental diets for 15 weeks. At the end of the trial, wild animals were harvested from the sampling site and compared with those fed the experimental diet.

Irrespective of the genre, the gonadal somatic index (GSI) of sea urchins fed the experimental diet was higher (20-23%) than average values observed in wild specimens (6-7%), with similar stage of maturation. Final gonad composition of sea urchins fed diet D30 had higher protein but lower lipid content, than their wild counterparts.

Gonads of wild males and females had significantly higher total carotenoid content mainly resulting from higher levels of echinenone,  $\beta$ -carotene and  $\beta$ -cryptoxanthin. Echinenone was the dominant carotenoid in gonads from both sexes, but males had higher levels in both wild and cultivated sea urchins (Table 1). Gonads redness (a\* value) was significantly higher in wild urchins, and females always exhibited reddish gonads compared to males. Wild specimens had firmer gonads than those fed the experimental diet, but resilience remained similar among animals.

The experimental diet significantly improved gonad size but resulted in gonads with a significantly different colour from wild specimens. Further studies are required to evaluate diets rich in pigments able to enhance gonads redness in farmed sea urchins.

**Table 1** -Gonads carotenoid content and colour in sea urchin male and female fed an experimental diet under captivity (D30) or harvest in the wild.

	Male		Female		Two-way ANOVA P-value		
	D30	Wild	D30	Wild	Genre	Diet	G x D
<b>Carotenoids</b>							
Total carotenoid ( $\mu\text{g/g WW}$ )	11.9 $\pm$ 3.9	89.1 $\pm$ 45.1	15.3 $\pm$ 1.0	86.6 $\pm$ 25.1	1.0	0.001	0.9
$\alpha$ -carotene	0.2 $\pm$ 0.01 <sup>c</sup>	1.3 $\pm$ 0.05 <sup>a</sup>	0.2 $\pm$ 0.02 <sup>c</sup>	0.4 $\pm$ 0.02 <sup>b</sup>	<0.001	<0.001	<0.001
$\beta$ - carotene	1.0 $\pm$ 0.02 <sup>c</sup>	19.4 $\pm$ 0.1 <sup>a</sup>	2.1 $\pm$ 0.01 <sup>c</sup>	10.1 $\pm$ 0.6 <sup>b</sup>	<0.001	<0.001	<0.001
Echinenone	22.0 $\pm$ 0.1 <sup>c</sup>	93.8 $\pm$ 0.2 <sup>a</sup>	8.0 $\pm$ 0.04 <sup>d</sup>	26.5 $\pm$ 0.01 <sup>b</sup>	<0.001	<0.001	<0.001
Lutein	0.2 $\pm$ 0.00 <sup>d</sup>	0.7 $\pm$ 0.01 <sup>c</sup>	1.8 $\pm$ 0.04 <sup>b</sup>	4.6 $\pm$ 0.04 <sup>a</sup>	<0.001	<0.001	<0.001
$\beta$ -cryptoxantin	5.1 $\pm$ 0.00 <sup>b</sup>	20.3 $\pm$ 0.01 <sup>a</sup>	4.6 $\pm$ 0.00 <sup>c</sup>	20.3 $\pm$ 0.01 <sup>a</sup>	<0.001	<0.001	<0.001
<b>Colour and texture</b>							
L* (Brightness)	59.0 $\pm$ 6.6	47.8 $\pm$ 4.7	46.3 $\pm$ 3.8	38.2 $\pm$ 4.0	0.004	0.01	0.6
a* (Redness)	7.1 $\pm$ 3.2	14.7 $\pm$ 2.9	1825 $\pm$ 0.2	25.4 $\pm$ 3.2	<0.001	0.001	0.9
b* (Yellowness)	25.1 $\pm$ 4.0	25.1 $\pm$ 0.5	34.3 $\pm$ 3.3	34.3 $\pm$ 5.5	0.03	1.0	1.0

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### Abstract

In recent years, seaweeds have gained recognition as a healthy food and became a new product in the gourmet industry. Additionally, their extracts are an important staple in a variety of industries, such as cosmetic, health, nutraceutical and food. This has been driving an increase in demand, risking the overexploitation of wild populations. This high demand drives an increase in seaweed value, motivating the development of efficient methods and practices for their production.

Northern Portugal is a region rich in seaweed species, many of which are of particular economic interest. However, to enable their commercial production, adequate systems and cultivation methods need to be developed, which must be specific for each species. With this in mind, four economically interesting species, with different environmental demands, were studied: *Codium tomentosum* (commonly known as “spongweed”); *Ulva intestinalis* (“sea lettuce”); *Laminaria ochroleuca* (“Atlantic kombu”); and *Osmundea pinnatifida* (“pepper dulse”). Results showed that nutrient addition were necessary to achieve high yields, as water renovation rates amounted to one renewal every two days. Nutrient demand varies throughout the year, according to seaweed growth rates, driving the need to adapt nutrient addition as to avoid either epiphytic overgrowth or growth limitation. Density is also an important factor, as it not only affects the amount of metabolites in the water and nutrient and carbon dioxide availability, but also causes self-shading, which may be an advantage for species sensitive to high irradiance, but a disadvantage for more light-adapted seaweed. Some species are also more resistant to temperature fluctuations and may cope well with exposure in an outdoor system, while other might be damaged by high temperatures, driving the need for strategies for stabilizing conditions, such as increasing the water renewal rate.

Cultivated seaweeds might also differ chemically from those harvested in the wild, depending on the cultivation conditions. For example, nutrient availability can be increased to achieve a more nutritious product, with higher protein content, or with higher content in bioactive compounds.

As nutrient addition increases production costs, seaweed cultivation in nutrient rich seawater, like fish farms effluents, allows for nutrient reuse, with environmental and economic advantages, concept which is known as Integrated multitrophic aquaculture system.

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### Abstract

Raw gonads of sea urchins have long been appreciated for their unique taste, particularly in Asian countries. Nowadays, other countries, such as different European countries, are incorporating such item in their gastronomic cultures, which makes sea urchins an option to consider in aquaculture. *Paracentrotus lividus* (Echinodermata: Parechinidae) is a common species in the littoral and sublittoral zones of the northeastern Atlantic and the Mediterranean. In view of avoiding species overexploitation, optimization of culturing conditions is required, namely with respect to preventing epizootic outbreaks associated with parasites. It is therefore crucial to evaluate potential parasite infections, both in qualitative and quantitative terms. Between May 2016-May 2017, samples of *P. lividus* were collected in three areas off northwest Portugal, northeast Atlantic waters - Praia Norte (PN; N=141), Carreço (C; N=112) and Vila Chã (VC; N=124). Parasitological examination of sea urchins allowed identification of different taxa: *Syndesmissaethopharynx* (PN, perivisceral fluid [prevalence=22%] and intestine [<1%]), *Syndesmis echinorum* (PN, perivisceral fluid [12%] and pharynx [<1%]; and C, perivisceral fluid [5%]) and *Syndesmis* sp. (C, perivisceral fluid [<1%]; and VC, perivisceral fluid [<1%]) (Rhabditophora: Umagillidae); *Asterocheresechinicola* (C, body surface [11%]; and VC, body surface [9%]), *Asterochereskervillei* (PN, body surface [1%]; C, body surface [11%]; and VC, body surface [7%]) and *Asterocheres* sp. (PN, body surface [<1%]; C, body surface [3%]; and VC, body surface [<1%]) (Copepoda: Asterocheridae); Trematoda (metacercaria) (C, water vascular system [54%] and Aristotle's lantern (muscles) [19%]; and VC, water vascular system [50%] and Aristotle's lantern (muscles) [19%]); Harpacticoida (C, body surface [13%]; parasitic?); and Amphipoda (C, body surface [9%]; and VC, body surface [17%]; parasitic?). Recorded infection levels suggest that parasites should not represent a major problem to be taken into account in culturing systems. Gonads of *P. lividus* were found uninfected, suggesting that their ingestion should not compromise human health.



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### Abstract

The current study evaluated the microalgae replacement by dry seaweed (*Ulva rigida*) in the reproductive success, biochemical composition and fatty acid profile of Pacific oyster (*Crassostrea gigas*) during broodstock conditioning. Two trials were carried out: a feeding trial for 11 weeks and a respirometry trial for 48 hours. Nine hundred oysters were divided in five dietary groups (3 tanks/diet; 60 oysters/tank). Each group were fed with one of the following diets: 100% seaweed (SW), 50%SW+50% live microalgae blend (Alg); 25%SW+75%Alg and 100%Alg. An additional group was kept Unfed during the entire feeding trial. The Alg diet was composed by 33% *Tisochrysis lutea* and 66% diatoms (75% *Skeletonema costatum* + 25% *Chaetoceros calcitrans*). Gonadal maturation was reflected in the physiological condition of the individuals. All treatments, except 100% SW, showed an increase in condition index and were fully matured at the end of the trial, with the best physiological condition observed in two groups: 75% Alg + 25% SW and 100% Alg. Conversely, oysters fed 100% SW showed a decrease in the reproductive condition and a delay in gonadal development. Protein and total lipids content increased during the conditioning, whereas glycogen decreased. Oysters fed 75%Alg+25% SW had higher protein and total lipids content and lower glycogen content (main energy reserve of gametogenesis) than the other treatments. In addition, this group showed the highest percentage of viable (free-living) veliger larvae after induction of spawning, even higher than the 100%Alg group. DHA (22:6n-3) and EPA (20:5n-3) contents showed a positive correlation with the percentage of microalgae in the diet, revealing a depletion and accumulation, respectively, coincident with spawning events. The poor FA composition of 100%SW led oysters to consume the initial FA reserves to overcome nutritional needs. The results of the respirometry trial showed an inhibition of oxygen consumption (OCR) and standard metabolic rate (SMR), suggesting a reduction of the metabolic rate in oysters fed 100% SW, which is in line with the condition index and gonadal maturation. The current study demonstrated that it is possible to replace 25% of microalgae with seaweed (*U. rigida*) in the broodstock conditioning of the Pacific oysters, which may enhance the operational cost efficiency in bivalve hatcheries.

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### Abstract

The interest in using marine organisms as a source of novel bioactive compounds has increased recently, reinforcing the need to find efficient and cost-effective methods to extract these biocompounds. Thus, the present study has focused on evaluating four solvent systems [methanol: water, 80:20 v/v (M80:20); methanol: water, 50:50 v/v (M50:50); ethanol: water, 80:20 (E80:20) and ethanol: water, 50:50 v/v (E50:50)] and three extraction methods [agitation at room temperature, agitation at 40°C, and ultrasounds] to obtain algal extracts of three macroalgae (*Gracilariasp. Fucus vesiculosus* and *Ulva rigida*) and two microalgae (*Chlorellasp.* and *Nannochloropsis gaditana*). The extracts were evaluated regarding yield, phenolic content and potential antioxidant activity, by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammoniumsalt (ABTS<sup>•+</sup>) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) radical scavenging assays.

Overall, no significant differences among the extraction procedures were observed; in contrast, differences were observed depending on the solvent system used. A lower organic solvent: water ratio increased macroalgae, but not microalgae, biomass extraction efficiency. Optimum levels of total phenolics (0.72 -16.3 mg GA/g DW), ortho-diphenols (0.79-19.69 mg GA/g DW), and flavonoids (0.28 - 7.38 mg CAT/g DW) were obtained with E80:20 and M80:20. Strong ABTS<sup>•+</sup> scavenging activity was obtained with E80:20 microalgal extracts, while no visible trend was detected for macroalgae extracts. On the other hand, methanolic extracts were the most effective in scavenging of DPPH<sup>•</sup>. Additionally, a high positive correlation was observed between macroalgae phenolic content and antioxidant capacity while in microalgae the opposite was verified, suggesting that phenolic compounds are not the main contributors to the observed antioxidant capacity.

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### Abstract

Aquaculture represents an environmental threat due to eutrophication caused by the generated organic rich effluents, also, the activity itself depends on a clean environment. The sustainable development of aquaculture requires the search for best practices and alternative solutions to reduce constrains (environmental and economic) posed by the generated effluents.

In aquaponics, where fish production is associated to hydroponic vegetable cultivation, primary producers act as extractive species, allowing the removal of the excess of nutrients from the effluents. However, regarding marine aquaculture, bioremediation of saline effluents implies the use of salt tolerant species, like halophytes. Furthermore, in light of the current circular economy agenda, preference is given to potentially commercial plant species. *Chenopodium quinoa* willd is a facultative halophyte known for its high nutritional value, rich in protein, essential amino-acids and antioxidants like phenols and flavonoids. The objective of this study was to test the effect of nitrogen (N) availability in the production of secondary metabolites, as a proxy for plant health status. The experiment was performed under controlled greenhouse conditions where plants were cultivated in hydroponic solution with a salinity of 20 and four nitrogen concentrations: 20 mg l<sup>-1</sup>; 40 mg l<sup>-1</sup>; 100 mg l<sup>-1</sup> and 200 mg l<sup>-1</sup>. After 4 weeks plants were characterized for biomass gain, ORAC, total phenols, total flavonoids, ascorbate, total N and elemental content. Nitrate concentration in the hydroponic solution was analysed for mass balance calculation. Results showed that, with a N concentration below 40 mg l<sup>-1</sup>, *C. quinoa* presents a higher antioxidant content but, at the cost of biomass gain, indicating a stress response. With a N availability above 100 mg l<sup>-1</sup>, plants biomass gain increased as well as the efficiency in N removal from the hydroponic solution. This study shows the potential of *C. quinoa* to be integrated in a saltwater facility (salinity of 20), in different production regimes, from semi-intensive to super-intensive.

**Keywords:** bioremediation; marine aquaculture; salt-tolerant plants; eutrophication; sustainable aquaculture.

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## Abstract

Sustainable aquaculture is highly dependent on the optimization of aquafeeds in terms of production costs, environmental sustainability and nutritional value. Senegalese sole (*Solea senegalensis*) is a prized flatfish produced in Europe with demonstrated capacity to cope well with both vegetable protein and oil sources. But the total replacement of fish oil in diets with a high inclusion of plant proteins has never been assessed before in this species. Three extruded isoenergetic (23 KJ/g) and isolipidic (15 % DM) practical PP-based diets (75% PP) were formulated: a control with fish oil (FO) and two experimental diets with 50% and 100% inclusion of a vegetable oil (VO) blend to replace FO (VO50 and VO100). Triplicate groups of fish (13.3±0.9g) were kept in a recirculation saltwater system at 19 °C and fed the experimental diets *ad libitum* over the course of 18 months. Fish were sampled after 6 and 18 months (in juvenile and on-growing development stages) to monitor weight gain and feed consumption. The total substitution of FO by VO (VO100) during the juvenile phase (6 months of feeding) significantly reduced final body weight, daily growth index, and protein gain, and increased FCR. A longer feeding period (after 18 months) showed that VO inclusion had no major effects on fish growth, but FCR was still significantly higher in fish fed VO100 than in fish fed the control diet at the end of the trial, muscle saturated FA level decreased with increasing dietary VO, whereas monounsaturated and polyunsaturated FA level increased in fish fed VO diets. Despite the lower values of DHA and EPA in the muscle with VO inclusion, the linoleic,  $\alpha$ -linolenic and arachidonic acid values significantly increased. Long term replacement of marine ingredients by vegetable oil and plant protein sources does not impair Senegalese sole growth, but strongly reduces EPA+DHA muscle levels compromising the nutritional value of those fillets for human consumption. A finishing diet able to restore the fillet fatty acid profile could be a good strategy to provide the recommended daily intake of omega-3 for healthy human individuals.

**Keywords:** Vegetable ingredients; Fish oil replacement; Senegalese sole; EPA/DHA

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### Abstract

Fish oil (FO) is an important aquaculture fish feed ingredient, but its limited natural supply has fostered the search for new lipid sources. The current study evaluated the capacity of tambaqui (*Colossoma macropomum*), one of the most produced fish species in Brazil, to biosynthesize long-chain polyunsaturated fatty acids (LC-PUFA) from dietary plant oil sources. To the best of our knowledge, little information is available about the effects of dietary vegetable oil (VO) levels on hepatic lipid metabolism in tambaqui. The current study evaluated the effects of different dietary lipid source and levels on the gene expression of key enzymes involved in LC-PUFA metabolism in juvenile tambaqui. Fish (IBW:  $8.61 \pm 1.38$ g) were fed for 9 weeks with four diets (FO5%, FO10%, VO5% and VO10%) and the gene expression of *fads2*, *elovl5* and *elovl2* were carried out in liver and brain tissues. The expression of *fads2* and *elovl5* in liver were upregulated when the fish were fed by the VO diets. It is plausible to infer that fish fed VO diet have more LA (18:2n-6) and LNA (18:3n-3) available for LC-PUFA biosynthesis. In addition, brain *fads2* and *elovl2* expression was up-regulated, with the higher activity of *elovl2* in the brain suggesting its important role in the biosynthesis of docosahexaenoic acid (DHA). In conclusion, the elongation and desaturation of C18 substrates are active processes for the production of LC-PUFA in tambaqui, when it is fed with a VO source in replacement of FO, showing that the tambaqui has the innate ability to biosynthesize LC-PUFA from C18 VO, thus satisfying its lipid essentiality for normal growth and development.

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# Poster presentations

## 1

## Effect of temperature fluctuation during an annual cycle on oxidative stress and energy allocation biomarkers of diploid and triploid *Oncorhynchus mykiss*

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### Abstract

*Oncorhynchus mykiss* culture is likely to be affected by the rising of water temperatures, expecting to stimulate fish metabolism, increasing oxygen demand and decrease energy available for growth. However, there is still uncertainty about the lower environmental tolerance of triploid fish compared to their diploid counterpart. The objective of this study was to assess the effects of water temperature on the oxidative stress status and cellular energy fractions of both diploid and triploid *O. mykiss* under culture conditions. Seven representative points of the annual cycle temperature fluctuation (6 - 22°C) were chosen. These samplings were performed in Quinta do Salmão (Portugal), from April 2016 to March 2017 in the same batch of fish. In each sampling, animals were anesthetized with MS-222 prior to dissection. Liver and muscle tissues samples were collected from 10 diploids and 10 triploid fish. Liver tissue was analyzed for lipid, sugar, protein, electron transport system (ETS) activity, lipid peroxidation (LPO), catalase (CAT), glutathione-S-transferase (GST) and total glutathione (TG). Muscle tissue was used for the analysis of energy reserves, and electron transport system (ETS) activity. Antioxidant defenses were significantly altered due to increases in temperature and differ between diploid and triploid fish. LPO levels were significantly reduced at higher temperatures. ETS activity decreased at higher temperatures, but a tendency for a higher activity was observed in triploid fish, which are in accordance with the known deficiency on oxygen delivery for these organisms. The different content of energy reserves on diploids underlines the differences on feed efficiency previously reported to diploid and triploid of other species. In muscle tissues, lipid content of diploids and triploids was differently affected by the lowest temperature. In sum, metabolic differences between diploids and triploids were observed in response of water temperature variation.

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### **Abstract**

The present study was designed to determine the modulatory effects of arginine and citrulline dietary supplementation on the immune condition and inflammatory response of the European seabass, *Dicentrarchus labrax*. Four diets were manufactured: a control diet (CTRL) was formulated to meet the indispensable amino acids profile established for seabass. Based on this formulation, three other diets were supplemented with DL-Arginine at two different levels (0.5 % and 1 % of feed, ARG1 and ARG2, respectively) and DL-Citrulline at 0.5 % (CIT). Fish were fed these diets for 2 or 4 weeks until visual satiation under controlled conditions. Blood samples were withdrawn from the caudal vessel at the end of each feeding period for the evaluation of the haematological profile. Blood smears were prepared for differential cell counting and total peripheral cells were counted as well. Blood was finally centrifuged and plasma samples were stored at -80 °C until assayed. Head-kidney tissue was also collected for the assessment of immune-related gene expression and kept at -80 °C until processed. At the same time, fish from all dietary treatments were intraperitoneally injected with *Photobacterium damsela piscicida* (5 x 10<sup>3</sup> CFU ml<sup>-1</sup>) and similarly sampled after 4, 24 or 48 h. Plasma peroxidase activity, total white blood cells and lymphocytes decreased with time before bacterial challenge regardless of dietary treatment, whereas lysozyme, total white blood cells, lymphocytes, monocytes and neutrophils decreased in fish fed supplemented-diets after infection. Expression patterns of a panel of 25 genes are currently being analysed and should further enlighten about immune mechanism in which arginine is involved. Nonetheless, results generally point at an impairment of both the immune status and inflammatory response upon dietary supplementation of either amino acid, a scenario that might result from nitric oxide toxic effects. Citrulline supplementation-induced changes were similar to those inflicted by arginine, suggesting that under immune stimulation, citrulline to arginine conversion rate might increase resulting in similar immune-impairment leading mechanisms.



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### Abstract

Similar to other stress factors, seasonal temperature patterns might lead to severe biological consequences such as growth impairment, delayed reproductive development and compromised immune performance. Nutritional strategies to enhance health and stimulate disease resistance are currently being adopted and amino acids have been shown to have immune-stimulating properties. Methionine (MET) metabolic pathways are also associated to key immune mechanisms such as cell proliferation and antioxidant activity. Differently, threonine (THR) is a major component of intestinal mucin and promotes antibody production in lymphocytes. Farmed rainbow trout (*Oncorhynchus mykiss*) is particularly exposed to thermic stress due to the wide range of temperature in inland water reservoirs throughout the year. The present study aimed at evaluating individual and synergistic effects of methionine and threonine dietary supplementation in rainbow trout exposed to increasing water temperature. Four diets were formulated based on a control diet (CTRL) which formulation met the amino acid requirement levels established for rainbow trout. To do so, MET and THR were added individually or in combination to final concentrations of 2 × the respective requirement level and were named MET2, THR2 and MT2. Dietary treatments were randomly assigned to triplicate tanks and fish were fed for 15 days. Water temperature was maintained at 15 °C for 6 days from which point it gradually increased to 24 over the next 9 days (1 °C per day). Fish were sampled at day 1 (T0-15 °C), day 7 (T1-16 °C), day 11 (T2-20 °C), day 13 (T3-22 °C) and day 15 (T4-24 °C). A control group fed CTRL was held at a stable temperature of 15 °C (CTRL15) throughout the feeding trial. Blood and head-kidney samples were collected at every time-point for cellular and humoral parameters and gene expression analysis, respectively. Preliminary results showed that lysozyme along with neutrophils, monocytes and thrombocytes increased with temperature, regardless of dietary treatment. When no signs of immune activation are observed, increased innate immune defenses such as lysozyme and phagocytes are indicators of a primed immune system. Since peroxidase plasma content did not increase above levels measured at the beginning of the feeding trial, the observed enhanced immune condition might have been the result of thermic stress-mediated priming effect. Despite the temperature-induced increase in cell numbers, neutrophil and lymphocyte percentages were lower in MET2-fed fish than in CTRL, which seems to suggest that methionine might be involved in negative feedback mechanisms to counteract the increasing leucocyte numbers. Despite no apparent effects of synergism or individual threonine were observed, molecular analysis are currently being carried out to evaluate possible genomic modulatory effects of these diets not detected at the metabolic level. Nonetheless, present results expose important aspects of methionine role in regulating stress and immune responses, highlighting its potential as a functional ingredient to farmed fish.

# 4

## Dietary tryptophan deficiency and supplementation compromises European seabass immune status and inflammatory mechanisms – a molecular approach

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### Abstract

Tryptophan catabolism plays a critical role in the immune mechanisms in macrophages mediating anti-microbial effects, regulating T-cell function and modulating cell oxidative status, favouring immune tolerance. However, the potential benefit of tryptophan supplementation for animal health management is not fully demonstrated. Therefore, the present study aimed to evaluate the effects of dietary tryptophan deficiency and supplementation on the European seabass immune status and inflammatory response through the evaluation of immune, tryptophan catabolism related genes, and disease resistance to *Photobacterium damsela piscicida* (*Phdp*).

Seabass juveniles were fed four experimental diets in triplicate tanks: control diet (CTRL); the CTRL diet supplemented with L-Tryptophan at 15 and 30 % above its requirement (TRP15 and TRP30, respectively); and a negative control diet (NTRP), deficient in tryptophan. Fish were sampled after 2 and 4 weeks of feeding and the remaining fish were injected with *Phdp* ( $5 \times 10^3$  cfu / fish) and sampled after 4, 24, 48 and 72 hours. Head-kidney was sampled for mRNA gene expression. The mortality of the remaining fish was recorded during 21 days.

In a tryptophan deficiency scenario, neither immune status nor the response to an inflammatory agent were altered, while disease resistance to *Phdp* was weakened. Moreover, a decrease in several pro-inflammatory genes was observed in fish fed TRP30 and in response to *Phdp*, eventually compromising at some level fish disease resistance.

In summary, results suggest that both dietary tryptophan deficiency and its supplementation at 30 % may compromise the European seabass inflammatory mechanisms and disease resistance.

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### **Abstract**

Rainbow trout (*Oncorhynchus mykiss*) farming faces several challenges in modern aquaculture, such as global warming, which may negatively affect health. Since methionine (Met) and threonine (Thr) present important regulatory roles in fish immunity, this study aimed to understand the combined effect of Met and Thr dietary supplementation in the rainbow trout immune status during exposure to thermic stress. A control diet (CTRL) was formulated to meet the rainbow trout AA requirements whereas two other diets were formulated to increase Met and Thr at 1.5 and 2 times their requirement level (1.5MT and 2MT, respectively). Fish were distributed over 24 tanks and fed the experimental diets in triplicates during a period of 2 weeks. Fish from 12 tanks were reared at constant 15 °C whereas fish from the other 12 tanks were submitted to a 10 °C increase in water temperature. At the end of the feeding period, fish (n=9) were sampled for plasma collection and further analyses of innate immune parameters. Plasma lysozyme activity showed no significant differences regarding diet or temperature, while anti-trypsin activity significantly increased with temperature, regardless diet. Peroxidase activity also augmented significantly with the increase in temperature, regardless dietary treatments. Moreover, fish fed 1.5MT and 2MT dietary treatments increased peroxidase levels, regardless temperature. Preliminary results suggest that both temperature and diet appear to activate an innate immune response in trout. Nonetheless, further analysis at molecular level will be assessed to better understand these results.

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### Abstract

Seasonal variations in both light duration and water temperature are known to be important factors that may affect fish immunity and disease resistance. The present study was thus conceived to evaluate the rainbow trout (*Oncorhynchus mykiss*) immunological status under farming conditions throughout a year. Blood samples were monthly collected at Quinta do Salmão (Pisões, Portugal) from two different groups (diploid and triploid fish) in a total of nine sampling points. In each sampling point, ten animals from each group were sampled and afterwards, at CIIMAR facilities, blood smears were performed, air dried and the rest of the plasma collected for humoral parameters quantification. Immune cells were identified, and a differential counts of neutrophils, monocytes, lymphocytes and thrombocytes were made. Humoral parameters in plasma such as peroxidase, lysozyme, protease and antiprotease activity were measured. Modulation of cells and humoral parameters were observed with seasonal variation. The relative number of peripheral lymphocyte and thrombocytes, as well as lysozyme and antiproteases activities increased in lower water temperature conditions. On the other hand, an increase in the relative number of circulating monocytes and neutrophils, together with an increase in plasma peroxidase activity, were observed with the augmentation of water temperature. No changes due to seasonal patterns were observed regarding the genetic background.

Although some of these variations may be caused by either fish normal development throughout the 9-month sampling period, genetic background and/or environmental interactions, the evaluation of more immune parameters, including IgM levels in plasma could bring more light to better understand the impact of seasonal changes in the rainbow trout immune status.

**Keywords:** rainbow trout, temperature, leukocytes, immune status, peroxidase, lysozyme, antiproteases.

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### Abstract

Though the measurement of molecular indicators of cellular stress (e.g. heat shock proteins, redox proteins) is prevalent in aquaculture research, and its importance is recognized in the context of assessing and managing animal stress, little effort is seen in distinguishing between adaptive (eustress) and maladaptive stress (distress) responses. This presentation explores a data-driven attempt at distinguishing between eustress and distress indicators, within the context of nutritional stress in gilthead sea bream. For this study, juvenile gilthead sea bream with a fishmeal diet background (IBW = 14g) were reared over the course of a 13-weeks feeding trial in 21 tanks (1000L), being fed one of the 11 different diets. Liver samples were obtained 2 weeks into the trial, and were used for the simultaneous profiling of a panel of 32 genes associated with different biological processes, as GH/IGF system and cellular stress. Results show that all indicators are strongly correlated (a sample that has a high value for one indicator tends to have high values for all indicators) and that the main difference observed is between 0% FM diets (higher levels of stress indicators) *vs.* diets containing FM (lower levels of stress indicators). On the other hand, a strong trend observed is that diets containing particularly low levels of crude lipid, induce the lowest levels of cellular stress (even lower than the ones observed for fish fed diets with 100% marine ingredients). Despite the generally high correlation between indicators, we have also observed that they can be clustered in 3 groups: general indicators of cellular stress, specific indicators of eustress (particularly higher for very high quality diets and diets with low energy) and specific indicators of distress (particularly higher for diets that either contained 0% FM or displayed very poor growth performance). To conclude, this study illustrates the importance of distinguishing between eustress and distress responses, and shows how one can infer this using a data-driven approach. In a practical sense, it underlines the challenge of going from low-FM to zero-FM diets without introducing nutritional stress, even when reasonable growth performance levels are displayed. Finally, the particularly lower level of all indicators observed for low lipid/low energy diets points towards a feasible nutritional strategy to mitigate hepatic stress before, during or after particularly challenging/stressful rearing situations (e.g. fish handling, fish transport, rearing under extreme temperatures).

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### Abstract

**Introduction:** Fish consumption is growing worldwide and therefore the aquaculture industry needs to cope with the high demand from society. Optimizing growth and fish health are important goals for the industry, so biological efficiency needs to be maximized. To improve fish growth and well-being, fish need to be fed with balanced diets, so usually amino acids are supplemented to fish diets. Methionine is an indispensable amino acid for normal growth of most animals, including fish, and it is required in the synthesis of cysteine and taurine, as well as being an essential methyl-donor in cellular metabolism. **Methodology:** In this trial, the impact of dietary methionine supplementation in European seabass juveniles was studied during 3 months. Juvenile European seabass with an initial body weight of 10 g, were fed with one of five experimental diets, which were formulated based on estimated requirements. Diet M0.65 had 0.65% (w/w) of methionine supplementation; diet M0.85, M1.25 and M1.5 had 0.85%, 1.25% and 1.5%, respectively of methionine. A FM (fish meal) diet was used as a positive control diet. Fish were fed twice a day, *ad libitum* and maintained in a flow-through system, dissolved oxygen in seawater was maintained above 5 mg L<sup>-1</sup> with a mean temperature of 22.4°C ± 1.9°C. Sampling was performed after 18 and 85 days, fish were lethally anesthetized with 2-phenoxyethanol, liver was collected and kept at -80°C for further analyses. Proteins were extracted using a DIGE buffer (7 M urea, 2M thiourea, 4% CHAPS and 30 mM tris, pH 8.8) and quantified by the Bradford method. Samples were subjected to a clean-up protocol to remove any contaminants. Pellets were resuspended in a lysis buffer (1% sodium deoxycholate, 10 mM TCEP, 40 mM CAA, 100 mM Tris pH 8.5), incubated for 5 min at 95°C and sonicated for 15 min. The shotgun proteomics protocol used was described in Kulak et al. (2014). **Results:** Fish growth results after 85 days of trial showed that FM fish had a specific growth rate (SGR) of 1.94 %/day followed by M1.5 fish (1.82 %/day), with a significant difference between M0.65 and the other three experimental diets (one-way ANOVA, followed by pos-hoc Tukey, p<0.05). Food conversion rate was the highest with M0.65 diet and the lowest with FM, 1.48 and 1.14, respectively, without any significant difference. Broken-line regression analysis of the zootechnical performance parameters, nonetheless, confirms the existence of a dose-dependent effect of methionine on growth performance. Analysis of protein expression using the semi-quantitative method for shotgun proteomics is ongoing.

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## Abstract

The feed management is important to assess the economic sustainability of sea urchins cultures. Therefore, it is important to define the feed ingredients and understand the nutrient intake. Nowadays, it has been a challenge to find the ideal feed for sea urchins, in order to obtain high quality and value of sea urchin roe for human consumption. It is necessary to have a nutritional balance in the feed composition, in order to attend the biological requirements of sea urchins. In this study, the effect of three formulated diets on the biochemical composition of sea urchin *Paracentrotus lividus* (Lamarck, 1816) gonads was evaluated. Sea urchins were collected in Peniche (western central coast of Portugal), with test diameter ranging from 30 to 45 mm. In order to promote a biological synchronization, sea urchins were starved for a month. After this period, they were fed twice a week (with jellified diets, representing 1.5% day<sup>-1</sup> of their average body weight), during 60 days. Diet A was formulated with maize (*Zea mays*) and New Zealand-spinach (*Tetragoniatetragonoides*); diet B with soya flakes (*Glycine max*), maize and New Zealand-spinach and; diet C with soya flakes and New Zealand-spinach. Recirculating aquaculture systems (RAS), with 60 L aquaria at 18 ± 1°C, were used in triplicate. Protein content of sea urchin gonads were assessed by the Lowry method (1951). For the determination of the lipid content, the Bligh and Dyer (1959) and De Coen *et al.* 1997 methods were used. Sea urchins fed with diet B had the highest values of protein and lipids (51.5 ± 2.3 mg g<sup>-1</sup> and 25.1 ± 3.4 mg g<sup>-1</sup> wet weight, respectively), when compared with diet A (37.3 ± 4.7 mg g<sup>-1</sup> and 19.7 ± 1.3 mg g<sup>-1</sup> wet weight, respectively) and diet C (33.8 ± 1.6 mg g<sup>-1</sup> and 21.2 ± 1.5 mg g<sup>-1</sup> wet weight, respectively). These results suggest that changes observed in gonad composition may be related to differences in the diets. Nevertheless, further studies are required to analyse the alterations of sea urchin gonads promoted by manipulating their diet.

**Key words:** echinoculture; nutrition; roe; protein; lipids; artificial feed

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### Abstract

Fish welfare is an essential matter in aquaculture, due to the fact that confinement and routine farm operations can be harmful to animals as they lead to significant amount of stress. To minimize stress, options are being explored, including the use of feed additives such as tryptophan (Trp). Trp is required for protein synthesis but also for synthesis of neurotransmitters such as serotonin and melatonin, which have a vital role in stress mitigation. Thus, this study aimed to assess the potential role of dietary Trp supplementation on growth performance and feed utilization of meager, *Argyrosomus regius* juveniles. Four isoproteic (45%DM) and isolipidic (16%DM) experimental diets were formulated: a control diet, with no Trp supplementation and three other diets including 0.5, 1, and 2 fold the Trp level of the control diet (diets 0.5Trp, 1Trp and 2Trp, respectively). Dietary tryptophan levels of the four diets were 0.49, 0.78, 0.95, 1.49 (%DM), respectively. The diets were tested in triplicate for 8 weeks. At the end of the trial a time-course blood sampling was performed, 6 fish from each dietary treatment were sampled at 1h, 3h, 6h, 12h and 24h after feeding. At the 6h sampling point, liver was also sampled. Results showed that the increasing up to 1 fold the dietary Trp level of the control diet did not improve growth performance, feed intake, or feed utilization. However, higher dietary supplementation decreased final body weight, weight gain, daily growth, feed efficiency, protein efficiency ratio, and nitrogen retention, but did not affect feed intake. At the end of the growth trial, whole-body composition, hepatosomatic and visceral indices were not affected by the dietary treatments, except for the 2Trp diet that decreased the whole-body lipid content. Irrespective of dietary Trp supplementation, the activity of amino acid catabolic enzymes was reduced when compared to control diet. Plasma protein was not affected by the experimental diets, but plasma triglycerides were higher in fish fed the control diet and plasma cholesterol was higher in 1Trp diet than the 2Trp diet. Moreover, Trp supplemented diets lowered plasma glucose levels in comparison to the control diet, suggesting that tryptophan can help in stress reduction. Overall, results showed that dietary tryptophan supplementation up to 1-fold higher than requirements may be beneficial in stress mitigation. However higher dietary supplementation levels seem to induce harmful effects, which may be related to tryptophan toxicity.

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### Abstract

The increased demand for sea cucumber species in Asian markets has led to a decline in Indo-Pacific fisheries. Fishing effort has, thus, intensified and expanded to areas of the Mediterranean, where different species are exploited. Farming of alternative species is now becoming crucial for the survival of natural stocks of these echinoderms. In general, this is a growing industry that demands the development and optimization of spawning induction protocols. *Holothuria tubulosa* has been seen as a new candidate for aquaculture, as this species is resistant to handling and adapts easily to captivity conditions. This study aimed to develop the successful artificial breeding and larval rearing of *H. tubulosa* and validate its potential as a new species for the aquaculture diversification. The broodstock of *H. tubulosa* was induced to spawn by thermal stimulation, raising the water temperature by 5° C. Spawning induction was successful. Males began releasing gametes thirty minutes after the temperature increased and the females began to spawn just after the males. The larval development of *H. tubulosa* progressed throughout five stages, reaching the pentactula stage in 27 days. The larvae were fed from the early auricularia stage with a mix of microalgae *Isochrysis galbana* and *Phaeodactylum tricornutum*, in equal proportion. The doliolaria stage was achieved after 25 days, setting the beginning of the settlement phase. In this stage the larvae fed on the biofilm formed in the tanks. The results obtained in this study indicate that *H. tubulosa* presents a high success rate in both posture and larval development. The settlement is a critical stage, with high mortality rates at this stage and there is still room for improvement regarding the settlement conditions (e.g. substrate, food, etc.). Nonetheless, this species is a valuable new candidate for aquaculture in the Southern Europe and in the Mediterranean region, both for production and restocking purposes.

**Keywords:** Sea cucumber, larval development, spawning induction, thermal stimulation, larval feeding

**Acknowledgements:** This work was financed by the Operational Program Mar2020. It received further financial support from Fundação para a Ciência e Tecnologia (project UID/MAR/04292/2013 and A.C. Brito with the Research Programme – IF/00331/2013).

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### Abstract

The present study focused on administrating three different diets in *P. lividus* throughout four months to assess their effect on the gonadosomatic index (GI) and on the development of the gametogenic cycle. Three jellified diets, using agar as a binding agent, were formulated as: diet A – maize (*Zea mays*) and spinach (*Spinacia oleracea*), diet B – maize, spinach and acorn (*Quercus ilex*) and diet C – maize, spinach and pumpkin (*Cucurbita maxima*). The sea urchins were first subjected to 44 days of starvation to promote gonadal reabsorption and to reset the reproductive cycle, after which they presented a GI of  $2.48 \pm 1.12\%$ . A higher GI was obtained from sea urchins fed with diet A ( $8.71 \pm 2.18\%$ ). Diet C promoted a final GI value of  $4.44 \pm 2.20\%$ , while the individuals from diet B exhibited a regression of their reproductive status ( $0.92 \pm 0.91\%$ ). Both diet A and C endorsed advances in the gametogenic cycle. Diet A led to 14% mature and 36% premature individuals, while diet C attained 28% mature and 17% premature individuals. Diet B resulted in a setback for the development of the gametogenic cycle, obtaining 100% of individuals in the spent stage. The results obtained in this study showed that the use of a mixture of maize and spinach as a dietary feed for *P. lividus* can improve gonadal growth and consequent gonadal maturation. However, it is still necessary to focus research efforts on feeding ingredients that improve gonadal growth without a quick maturation of the gonads.

**Key words:** echinoculture; roe enhancement; aquaculture; jellified diets; nutrition; reproductive cycle.

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### Abstract

Dissolved carbon dioxide (dCO<sub>2</sub>) evaluation is very important in many different fields as ocean, river and lake monitoring, food industry control (e.g. aquaculture) and clinical analysis. So far, there are some different methods for CO<sub>2</sub> measurements, including gas chromatography, colorimetry, amperometry, potentiometry, UV/Vis spectrophotometry and IR spectrometry [1]. These methods are time-consuming, expensive and usually not suitable for real-time monitoring of dCO<sub>2</sub>. In the last years, several authors developed optical fiber-based technology for real time detection and quantification of dissolved carbon dioxide [2-5]. In this work, it was developed a new, integrated, colorimetric-optical fiber-based system for dCO<sub>2</sub> monitoring in the aquaculture industry. The sensing chemistry is based on a colorimetric indicator - *p*-nitrophenol (pNPh). Poly-pNPh derivatives were used to increase *p*-nitrophenol pK<sub>a</sub> range (pK<sub>a</sub> = 7.2) and thus the sensitivity of the sensing membrane. The resulting sensing layer was attached to an optical fiber probe in transmission mode illuminated with integrated dual wavelength LED. In the presence of carbon dioxide, the sensing layer changes its optical properties (absorption and refractive index), which changes the posterior optical response. This response is analyzed by a computer software specially developed for this purpose. Preliminary tests were done in a laboratory environment and in a laboratory fish culture system with water recirculation. The results showed the suitability of the new sensor for assessing dCO<sub>2</sub> dynamics and its fast detection of low concentrations of dCO<sub>2</sub> in an appropriate operation range.

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### Abstract

The sea cucumber is an Echinoderm with high economic interest. Due to increasing demand, the heavy pressure imposed on this group is so such that its natural stock is dwindling worldwide. A steep decrease in abundance of wild sea cucumbers has been observed, which together with the rising interest in consumption from the general public has lead to a high stakes arms race to grow and produce sea cucumbers in captivity.

This study aimed to determine the suitability of maintaining a broodstock collection of *H. forskali* in captivity regarding the maturation of their gonads and future prospects for induced spawning. *H. forskali* individuals were collected in January from the marine coastal area of Arrabida, Portugal, and maintained in captivity over a period of five months, separated into two different stock densities: 15 kgm<sup>-3</sup> and 30 kgm<sup>-3</sup>, in order to ascertain the influence of stock density in captivity. Water parameters were monitored daily to ensure good water quality throughout the study. The mean temperature recorded was 17.97 ± 1.04 °C for the lower density and 17.99 ± 0.91 °C for the higher density. Mean Dissolved Oxygen (DO) and salinity were observed as 7.04 ± 0.41mgL<sup>-1</sup> and 33.31 ± 0.86 psu, respectively for the lower density whereas the higher density presented 6.89 ± 0.42mgL<sup>-1</sup> and 34.12 ± 1.10 psu for the same parameters. Both densities presented a mean pH value of 8.35 ± 0.07. Neither of the densities in question presented harmful levels of ammonia or nitrites. Individuals maintained in captivity, in either density, presented no statistically significant differences regarding their Gonadosomatic Index when compared to wild individuals collected at the start of the trial whereas wild individuals collected at the end of the trial did present a significantly smaller GI according to the same test.

These results point towards the positive effect of captivity regarding the breeding of this species, in particular over the fact that individuals kept under these conditions are able to outlast the species' natural spawning period.

**Keywords:** *Holothuria forskali*, spawning, Gonadosomatic Index, stock density, broodstock collection

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## Abstract

European seabass is an aquaculture species with economic interest whose production depends on fishmeal-based diets. However, fishmeal (FM) is an unsustainable commodity that must be replaced with new sustainable ingredients, such as macroalgae. Furthermore, macroalgae are also an important source of important bioactive compounds. However, macroalgae nutrient bioavailability is limited due to its cell wall, which is undigested by fish. Thus, it was hypothesized if the cell wall degradation, through physical, chemical or biological treatments, may increase its biological value. Macroalga (*Ulva rigida*) was pre-treated through a physical (ultrasound), chemical (alkali) and biological (solid-state fermentation (SSF) and enzymatic digestion) processes. Seven isoproteic (45% crude protein) and isolipidic (18% crude lipids) diets were formulated. A 25% FM based diet without the incorporation of *Ulva rigid* was used as a control. The other diets were formulated with 17.5%FM and including 5% of *Ulva rigida* untreated or treated with the aforementioned pre-treatments. Triplicate groups of 15 on-growing sea bass (mean initial body weight  $\pm$  107g) were fed with these diets by hand, to apparent satiation, two times a day, six days a week, for 8 weeks, at 24 °C. At the end of the growth trial, fish were bulk weighted, two fish per tank were collected for whole-body composition analysis and the blood of three other fish per tank was collected for plasma analysis. Growth performance of FM-control diet and SSF algae treatment were similar and higher than that of enzymatic hydrolyze treatment. The inclusion of both untreated and pre-treated *Ulva rigida* decreased feed intake. Feed efficiency was highest with the SSF treatment diet and lowest with the enzymatic hydrolyze treatment. At the end of the growth trial, the whole-body composition was not affected by dietary treatments. Likewise, plasma glucose, triglycerides, and total protein levels were not affected by the inclusion of untreated and pre-treated *Ulva rigida*. Though, the inclusion of untreated algae, ultrasound, and alkali pre-treated algae increased plasma cholesterol levels, whereas SSF pre-treated algae decreased cholesterol up to levels observed with the control diet. In conclusion, reduction of FM from 20 to 17.5% with the concomitant inclusion of SSF treated *Ulva rigid* did not affect growth performances, while with the inclusion of enzymatic hydrolyzed *Ulva rigida* it was reduced. Algae alkali and SSF pre-treatments resulted in the highest feed efficiency. The increase in plasma cholesterol levels suggests that further studies are necessary to evaluate the effect of macroalgae on fish metabolism.

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### Abstract

*Spondyliosoma cantharus* (Linnaeus, 1758) or black seabream, it is a much appreciated marine fish species in Portugal. Due to this, a study to evaluate their parasitofauna was conducted with aim to also study its distribution according to host ontogeny. A total of 39 fish were collected in Aveiro's North Atlantic coast, in September and October 2017, characterized according to weight, length, age, and sex and sexual maturation, and a parasitological survey was performed.

Younger fish, holding mainly 1+ or 2+ years old, measured and weighted (mean±SD) 21.6±1.0 cm and 176.9±25.6 g (n=19), while older fish, holding mainly 3+ or 4+ years old recorded 27.5±1.8 cm e 389.9±57.9 g (n=20). Parasitological indexes such as prevalence, intensity and abundance were determined for each identified taxon of each fish group. The parasites recorded for the young and the older fish were (with Prevalence in % / mean Abundance / mean Intensity): 2 Monogenea- *Bivagina alcedinis* (42.1% / 0.5 / 1.3 and 80.0% / 2.6 / 3.3) in gills, and *Choricotyle chrysophrii* (10.5% / 0.1 / 1.0 and 15.0% / 0.4 / 2.3), in buccal cavity; 1 Cestoda larva (5.3% / 0.1 / 1 and 0% / 0 / -) in intestine; 1 Nematoda (10.5% / - / - and 0% / - / -) in gonads; 1 Copepoda: *Alellapagelli* (42.1% / 0.5 / 1.4 and 10.0% / 0.4 / 4.0) in gills; Amphipoda (0% / 0 / - and 15.0% / 0.4 / 2.3) in gills; and Tanaidacea (5.3% / 0.3 / 6 and 15.0% / 0.2 / 1.0) in buccal cavity. The comparison among the parasitological records of the two groups of fish recorded only significant differences for the occurrence and abundance values of *B. alcedinis* and of *A. pagelli*, with Qui-square values >5.3 and P<0.02, and Man-Whitney U values <132.0 and P<0.03.



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### Abstract

European pilchard (*Sardinapilchardus*) is among the top 5 most sold fish in Portugal and in other European countries. Besides its high economic value, few parasitological studies were performed so far. In a recent parasitological survey, it was recorded that it holds only a few macroparasites. It was completely depleted of ectoparasites, and as endoparasites, only 2 worms of *Radinorhynchus* and 818 anisakid worms, from 230 adult fish analysed, were recorded. Another sample of 40 juvenile fish was also surveyed, but they did show no macroparasites, ecto or endoparasites. For anisakid infection, the minimum size of host infected was 14.9 cm, which is also when the fish achieve its sexual maturity, and probably when its diet changes, turning it susceptible to anisakid infection. The fisheries ground localities show also to be related with infection levels of anisakids. Higher infection was detected in FAO zone 27, subarea II/VII, medium in subarea VIII, and lower in subarea IX, what seems to represent a decrease correlated with a decrease of latitude, probably related with anisakids definitive and first intermediate host availability in these localities. In terms of seasonality, in autumn and winter months fish exhibited higher anisakid infection levels and in spring and summer months they exhibited lower levels. This variation is most probably related to the food ingestion quantity that achieved higher values in cold months, where fish spend almost all energy in gonad development, for achieving sexual maturity. This will happen in hot months, where the fish eat less quantity of food items and are less infected.



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### Abstract

Some *Kudoa* species, including *Kudoa thyrsites*, cause post-mortem myoliquefaction in heavily infected hosts rendering affected fishes unacceptable for commercial purposes. In the present work 162 European pilchards (*Sardina pilchardus*) from Portuguese (Aveiro and Matosinhos) and Spanish (Rías Baixas) Atlantic waters were analyzed for the presence of *Kudoa* myxospores which were morphologically, morphometrically, and molecularly characterized. The characteristics of myxospores agreed with those of *Kudoathyrsites*, a problematic taxon reported from many other host species which could include some cryptic species. In addition, the analysis of their SSU and LSU rDNA sequences also revealed a very close relationship with *K. thyrsites* (100% and 99.7% respectively). The prevalence of the parasite in mature fishes from Aveiro, Matosinhos and Rías Baixas waters (all of them belonging to the ICES IXa division) was in all cases higher than 50% (55%, 77.5% and 59.4% respectively). Infection parameters were clearly related to host size, with spores being detected only in fishes measuring more than 16.4 cm in fork length. Our results therefore indicate that *Sardina pilchardus* is a new host for the species *K. thyrsites*.

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### Abstract

When arriving to a new locality, invasive host species, generally leave their parasite fauna behind, and get a new set of parasites. In between, a stage without major parasites, with advantages to the invader success, can be achieved. The parasitological state of Pacific oysters (*Magallanagigas*) produced at two different estuaries in Portugal, one in the North of Portugal, at Lima Estuary, and another in the Centre, at Aveiro Estuary, was determined.

141 oysters were analysed from Aveiro Estuary, presented (mean±SD (minimum-maximum) total weight=72.5±15.5 (46.5-128.8) g, shell length = 9.2±1.2 (5.1-12.5) cm, 63 males and 78 females, and the parasite fauna recorded was: *Mytilicola intestinalis* (Prevalence - P: 1.4%, Abundance (mean±SD, maximum) - A: 0.04±0.35, 4) in digestive tube; *Mycicola* sp. (P=1.4%, A=0.01±0.12, 1) in border of mantle; *Ostrincola* sp. (P=0.7%, A=0.0±0.08, 1) in the gills; *Trichodina* sp. (P=2.8%, A=0.07±0.50, 5) in the gills, labial palps, and gonads. While, from Lima Estuary, 150 oysters were analysed, presented total weight = 60.1±21.2 (18.2-126.9) g, shell length = 8.9±1.7 (5.2-14.2) cm, 82 males and 68 females, and the parasite fauna found was null.

One explanation for these different parasitological findings may be the different availability of freshwater in the two locations. Actually, freshwater is present twice a day, with low tides in Lima, and is absent in Aveiro, which is much closer to the ocean. The constant salinity of the later, allows the installation of a diverse parasite fauna, that kept in low levels (as the ones found) are not troublesome to oyster production, but if elevated might cause significant damage to the hosts. Moreover, Crustacea and Ciliophora have usually high reproductive potential, so the oysters should be under surveillance for parasite levels. Also, due to its high mobility, these parasites can be easily transmitted to native oysters with harmful consequences.

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### Abstract

*Diplodus sargus* Lineus, 1758 (Sparidae) is a highly appreciated fish in Portugal and European countries and its parasite fauna is poorly known. The aim of our work was to characterize its ectoparasite fauna, to observe the pattern of distribution of each species inside gills, and to analyse possible interspecific interactions. 30 fishes from a local fish market were examined for gills' parasites. Several different microhabitats within the holobranch were established (proximal, medial and distal; internal and external; and anterior and posterior hemibranch). A total of eight macro ectoparasites species/taxa were found in the gills: four of them were Monogenea: *Atristasterheterodus* Lebedev & Parukhin, 1969, *Atrispinum seminalis* Euzet&Maillard, 1973, *Diclidophora* sp.; and *Lamellodiscus echeneis* (Wagener,1857); from which the two first Microcotylidaewere the most prevalent (P=63.3%); while *Diclidophora*sp. record only 6.7%; other three parasites were Copepoda (*Clavellis*asp.; *Hatschekia pagellibogneravei* (Hesse, 1878),and *Lernaepodidaegen.* sp., with *H. pagellibogneravei* achieving P=63.3%; and one Isopoda (*Gnathiasp.* P=10.0%). The fish total length and total weight shown a positive correlation with *L. echeneis*abundance. In terms of gills distribution only *H. pagellibogneravei*abundance showed preference for the gills of the right side, medial segment, and for anterior hemibranch. Regarding the interspecific relationships of the parasites, the results show a significant negative correlation between the pairs *H. pagellibogneravei/L. echenis*and the pairs *H. pagellibogneravei/A. heterodus+A. seminalis*. The numerical response analysis detected differences between singular versus concomitant abundance infections for the pair *H. pagellibogneravei/L. echneis*. This study confirmed the existence of an interspecific competition, between *L. Echneis* and *H. pagellibogneravei*, with the dominance of the former.

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### Abstract

The genus *Scomber* Linnaeus, 1758 is the most representative of the Scombridae family, with migratory and epipelagic fish with elongated and fusiform body. The species *Scombercolias* Gmelin 1789, is distributed in the North Atlantic Ocean and Mediterranean Sea. The aim of the authors with this study were to determine the existence of a distribution patterns by microhabitat of branchial Monogenea parasites, in Atlantic chub mackerel *S. colias* of the coast of Portugal.

In May and July 2018, a total of 50 Atlantic chub mackerel specimens were collected for the characterization of the parasite fauna and its microhabitat in the gills. After the biometric characterization of the fish, the gills were carefully removed and the gill chambers separated into Petri dishes and divided according to the left and right sides, and numbered from the outer region to the inner of the operculum, such as holobranchs I, II, III and IV. Additionally, each holobranchs was separated in three proximal, medial and distal segments, and in two anterior and posterior hemibranchs; and each hemibranch in internal and external sites. The parasites Monogenea *Pseudokuhnia minor*, *Kuhniascombri* and *Grubea cochlear* were recorded. *P. minor* was the most prevalent (P = 96%) followed by *K. scombri* (P = 48%). There was no significant correlation in the comparisons between host - parasite nor between pairs of parasite species. *P. minor* presented a preference for holobranch I, II, III and the proximal segment for abundance and occurrence (Friedman test:  $\chi^2 = 16.59$ ,  $p = 0.00$ ; Cochran test:  $Q = 9.75$ ,  $p = 0.008$ , respectively). *K. scombri* showed preference for the interior of the gills for abundance and for occurrence (Wilcoxon test:  $Z = -2.900$ ,  $p = 0.003$ , McNemar test:  $p = 0.011$ , respectively), and holobranch I, II, III for abundance and occurrence (Friedman test:  $\chi^2 = 13,095$ ,  $p = 0.004$ , Cochran test:  $Q = 13.83$ ,  $p = 0.003$ , respectively). Although the statistical result showed no interspecific correlation between *K. scombri* and *P. minor*, both showed preference for the same microhabitat, holobranch I, II, III in this study.

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### Abstract

Aquaculture systems need to optimize water resources to cope with sustainability and environmental issues. In land-based aquaculture, this can be achieved through the utilization of Recirculating Aquaculture Systems (RAS), where water is recycled after treatment. Toxic ammonia and nitrite are transformed by nitrification in Biological Filters (BFs), which are a vital part of the water purification system. The Moving Bed Biofilm Reactor (MBBR) is a type of BF used in RAS where bacteria are developed in supports moving freely in the reactor. Understanding MBBR behavior under different RAS operational scenarios is fundamental for optimal RAS functioning.

In this study, the effects of changes in water recirculation rate on MBBR performance were investigated in a laboratory trout (*Onchorynchus mykiss*) RAS system ( $V=1\text{ m}^3$ ). Three test periods of 2-weeks were followed: normal or Before Disturbance (BD), increased or During Disturbance (DD, 50% increased RAS) and recovered or After Disturbance (AD). MBBR behavior was assessed through changes in biofilm development (gravimetry), total bacterial cells (DAPI staining), physiological response (closed respirometry, nitrification potential) and prokaryotic community structure – (16S rRNA gene massive sequencing, on-going). System water quality was evaluated daily for routine maintenance and more intensively once at each test period, before and after feeding the fish. Fish growth and mortality were also determined.

Results show that the increase in RAS applied led to an increase in system organic matter and ammonia and a decrease in pH. MBBR bacteria were highly impacted by the disturbance applied. In fact, biofilm biomass and total bacterial numbers decreased and TAN (Total Ammonia Nitrogen) removal efficiency dropped. Biofilm oxygen uptake rate decreased also denoting stress. Nevertheless, in the recovery phase (AD), biofilm biomass, cell numbers, and oxygen uptake rates increased, and TAN removal capacity tripled, when compared to initial (BD) values. Fish mortality was null and a 58% fresh weight increase was observed during this experiment.

Data obtained show that operational changes like an increase in RAS affect biofilter bacterial community at different levels, but also suggest that such changes are not detrimental to the biofilm and should be further investigated.

**Keywords:** Aquaculture, RAS; MBBR, biofilm, stress, nitrification, bacterial compounds

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### Abstract

In the search for a healthier way of life, Europeans are increasingly recognizing seaweeds as a healthy food. In addition to its applications as food, seaweed are also used as food supplements, nutraceuticals, biomass for biofuel and feed for animals. With this increase in demand, the risk of overexploiting the natural resources is very high. As such, developing sustainable alternatives of producing biomass is crucial. *Codium tomentosum* is an abundant specie in Europe. Several studies highlight not only its nutritional potential for human food but also its versatility as a source of diverse bioactive compounds with interest for the cosmetic and pharmaceutical industries. In this project, cultivation methodologies for *Codium tomentosum* were studied. As a first step, trials in the laboratory were performed to determine the best temperature (12°C, 16°C and 20°C), density (5, 10, 15 g/L) and light intensity (100 and 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for biomass production. The best results were obtained at 16°C and 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ , with no significant differences between the 3 densities tested. This seaweed was able to grow at all temperatures, as expected of a temperate seaweed. Alongside these controlled experiments, this species was also cultivated in an outdoor cultivation system during 2 different seasons of the year (winter and summer). In winter (the slow growth season) *Codium tomentosum* presented a positive growth rate for most of the time and few epiphytes were present, whereas in summer (the fast growth season) growth was observed during the whole trial. Despite the faster growth of this seaweed in summer, this was also accompanied by overgrowth of other green seaweed in the tanks, which compete with *C. tomentosum* for light and nutrients. The biochemical analyses of this seaweed showed that the cultivation process didn't affect the values of antioxidant activities and the content in protein and improved the phenol content. These results indicate that the cultivation process might not negatively affect the components studied and, in the case of phenols, might even increase their production. In conclusion, *Codium tomentosum* is a specie with potential for aquaculture. Further studies are, however, still needed to better understand the best conditions for the cultivation of this seaweed.

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### Abstract

A successful seaweed culture system requires a flawless knowledge of the target species, in order to achieve effective and profitable growth and production rates. In this sense, culture methodologies have been currently researched, improved, and implemented in culture systems worldwide dedicated to seaweed growth. The red seaweed *Gracilaria gracilis* is extensively acknowledged both as anagrophyte and as a food and feed natural component, therefore being widely researched and cultivated worldwide. In the present work, *G. gracilis* growth rates, protein and lipid content of specimens kept in controlled culture conditions were evaluated and compared to wild specimens. Healthy specimens of *G. gracilis* were harvested during low tide, in Lagoa de Óbidos (LO: 39°24'18.93"N, 9°11'13.05"W) and Figueira da Foz (FF: 40°07'56.5"N, 8°50'35.9"W), Portugal. In the lab, all specimens were individually washed and thoroughly cleaned; a portion of the biomass was stored at -20 °C until biochemical analysis, and the remaining portion was placed under acclimatization for one week in dark containers provided with clean saltwater (20°C, 30-35 psu, 16:08 Light:Dark). Afterwards, the acclimatized biomass was distributed across three distinct containers: 50 L semi-transparent tanks, Petridishes, and 250 mL flat-bottom flasks, in climatic rooms set at 20°C. The light provided to the seaweed placed in tanks was supplied by white cool light bulbs with photoperiod set at 16:8 L:D, whereas the light provided to the seaweed placed in Petri dishes and flat-bottom flasks was provided by distinct bulbs: white cool light (1500 lux), and a grolux plus daylight combo (1500 lux and 3000 lux). All individuals were provided with seawater at 35 psu, supplemented either twice a week (tanks), or on a weekly basis (Petri dishes), or on a fortnight basis (flasks) with Von Stosch Enriched (VSE) medium modified for red seaweeds (Redmond et al. 2014). All assays were performed in triplicate and individuals were weighted whenever culture media was renewed. Daily growth rates were calculated according to Hayashi et al. (2011). After four weeks, the seaweed kept in semitransparent tanks was dried at 25 °C, powdered, and stored until further analysis.

Protein content for both the seaweed grown in tanks, and wild specimens collected throughout the year, was assessed according to the Kjeldahl method (Kjeldahl 1883) with a conversion factor of 4.95, whereas total lipid content was evaluated by the Filch method (Filch et al. 1957).

*G. gracilis* from Figueira da Foz and Lagoa de Óbidos kept in culture conditions for four weeks showed distinct growth rates according to the type of container used. In semi-transparent tanks, the seaweed showed decreasing growth rates throughout the four weeks, being the highest value



found after the first week (3.3 and 3.0 %·day<sup>-1</sup> for LO and FF populations, respectively) and the lowest at the fourth week (0.1 and 0.5 %·day<sup>-1</sup> for LO and FF populations, respectively). Thus, this result showed the influence of biomass density on the optimal growth of this seaweed in closed systems. *G. gracilis* grown in flat-bottom flasks under the gro-lux and daylight combo light sources at 1500 lux showed the highest growth rates (the maximum value achieved was 17.50 % day<sup>-1</sup> for LO measured after the first fortnight). Results also demonstrated that both wild and cultivated *G. gracilis* exhibit a favourable nutritional biomass profile. Total protein content found in wild populations ranged from 6.70 ± 0.31 to 23.24 ± 2.22 (% in dry weight), being the highest protein content generally found within the autumn and winter season. Total lipid content ranged from 1.23 ± 0.14 to 5.6 ± 0.14 (% in dry weight) in wild specimens, being the highest lipid content found during the autumn season. *Gracilaria* specimens kept in controlled culture conditions presented higher protein content (36.45 ± 0.96 % in dry weight) and lower lipid content (1.65 ± 0.12% in dry weight) compared to wild *Gracilaria* populations. This high protein and low lipid content found in wild specimens of *G. gracilis* stand in agreement to what is usually reported for the species, genus, and red seaweeds in general (Lordanet al. 2011; Francavilla et al. 2013). The high protein and low fat content achieved for specimens kept in controlled culture conditions when compared to their wild counterparts are significant reasons to consider the large-scale culture of this seaweed in controlled aquaculture systems, in order to efficiently produce biochemically valuable specimens.

**Keywords:** *Gracilaria gracilis*, seaweed cultivation, growth rates, light intensity, nutritional profile.

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### Abstract

The effects of three different microalgae diets were assessed on the survival and growth of the purple sea urchin *Paracentrotus lividus* larvae. Diet A was composed equally by *Phaeodactylum tricornutum* and *Isochrysis galbana*, while Diet B consisted on *P. tricornutum* and *Tetraselmis chuii*, and Diet C was a mixture of *P. tricornutum*, *I. galbana* and *T. chuii*. Feeding was initiated when the larvae reached the 4-arm pluteus stage. The amount of microalgae cells provided was adapted according to the larval stages. The development of the embryonic and larval stages (blastula, gastrula, prism and pluteus larva) was observed and followed. The differences in survival and total length between larvae fed with the different diets were registered for a period of 10 days after fertilisation.

Results showed that Diet A promoted better results in growth performance and survival of pluteus larvae, achieving the early 6-arm pluteus stage in 9 days. In a parallel trial, Diet A was used to induce larval settlement, in which competent larva were exposed to three different substrates: oyster shell, corrugated polycarbonate sheets and brick. During larval development, the diameter of the larval rudiment was registered every third day from the 12th day post-fertilisation. Oyster shell promoted the highest settlement percentage of metamorphosed larvae (58.33%), followed by brick (33.33%) and corrugated polycarbonate sheets (8.33%).

The results highlighted that an optimized larval nutrition, associated with adequate settlement substrates, is crucial to improve *P. lividus* larval performance and settlement.

**Keywords:** purple sea urchin, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Tetraselmis chuii*, larval rearing, aquaculture

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### Abstract

Cyanobacteria are common components of the freshwater phytoplanktonic communities. Their presence can be associated with the production of toxic secondary metabolites, named cyanotoxins, with hepatotoxic, cytotoxic, neurotoxic and dermatotoxic effects in both humans and other animals. The exploitation of freshwater systems increase the presence of cyanobacteria in water, forming blooms, degrading its quality and exposing aquatic and terrestrial organisms to major hazards. Besides the decrease in water quality, the intensification of cyanobacterial blooms can also lead to major impacts in aquaculture, such as an increased degradation of aquaculture systems, the increased spread of diseases among the cultivated organisms, bioaccumulation of toxins and also to high mortality rates. For that reason, monitoring of water quality in freshwater systems used for aquaculture is extremely important. The development of methodologies that can detect and quantify the potential for production and toxicity of several cyanotoxins not covered by the legislation in force can be essential for a safe implementation of aquaculture procedures and systems. So, the main objective of this study is to point out the effects of toxic cyanobacterial blooms and the cyanotoxins occurrence in aquatic organisms and the impact of these in aquaculture, as well to inform about the current methodologies to assess these occurrences in the aquaculture systems.

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### Abstract

*Paracentrotus lividus* is the most common sea urchin in European waters. Although it is a species with a key role in the maintenance of the ecosystems, there is a lack of information regarding its major diseases and even in the knowledge of its immune system. To contribute to this endeavour, a challenge with *Vibrio anguillarum* was done. Three control and 3 experimental tanks with 10 *P. lividus* each ( $7.1 \pm 0.6$  cm;  $123.6 \pm 21.1$  g) were used for a bath challenge with  $3 \times 10^{12}$  bacteria/L. After 4 hours incubation, individuals were sampled for extraction of the coelomic fluid (CF) through the peristomial membrane. CF was then centrifuged and coelomocytes were separated and lysed (CL) to measure the major immune parameters. Total and differential cell counts, phagocytic activity against *Saccharomyces cerevisiae*, total protein content, lysozyme concentration, protease activity, nitric oxide production (NO) and RNA concentration were analysed.

Results showed no differences in coelomocyte concentration, being petaloid cells the most abundant immune cells of sea urchin. Several immune parameters changed in response to bacteria but with no significant differences with exception of NO content in CF, which indicates a host response. Total RNA expression also exhibited a clear trend to increase in response to bacterial challenge. Results suggest that 4 hours could not be enough time to show a clear response of the immune cells to an infection, but the increase of NO and RNA contents suggest an early response to bacterial challenge. Immunerelated genes will be identified after transcriptome analysis.

S. Brito, T.R. Pereira, I. Azevedo, I. Sousa-Pinto

### Abstract

In Europe, interest in seaweed, branded as healthy food and as a valuable source of bioactive compounds, has been increasing steadily during the past decade. *Ulva intestinalis* one of the most common genus of seaweeds in marine and brackish environments in the world. This species has several applications with potential for the European market, including cosmetics, medical use and food source, being even able to constitute a novel food for the crescent blue market. Additionally, its great capability of propagation, due to an opportunistic behaviour, constitutes a compelling factor that add to the interest in this species. The increasing biomass demand creates a need to develop sustainable methods of cultivation of this species, for which production optimization knowledge is necessary.

Laboratory trials were conducted to study which conditions (temperature, light, density) are best for the species. A first trial tested the effect of three different temperatures (12, 16 and 20 °C) on the growth of *U. intestinalis*. The species demonstrated higher growth at 16 °C, and sporulated the most at 20 °C, suggesting temperature is one of the factors that influence sporulation. The second trial tested the effect of three densities (5, 10 and 15 g/L) at two different photon fluency rates (100 and 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Growth was significantly higher at 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the first three weeks of the trial but caused higher sporulation. In the last two weeks, growth at both photon fluency rates did not show significant differences.

Furthermore, an assessment of the growth and biochemical composition seasonality of the algae, cultivated in a tank system in two distinct seasons: slow-growth season (winter) and fast-growth season (spring), was also performed. In the slow-growth season trial, *U. intestinalis* demonstrated an opportunistic behaviour, tending to sporulate when light intensity was low and to grow when light exposure increased. In the fast-growth season trial, temperatures caused sporulation in the same light levels in which higher growth occurred in the slow-growth trial. This suggests an interaction between the two factors. Biochemical results were inconclusive in determining the best time to harvest *U. intestinalis* and, overall, tank cultivation seems to have had little influence on the biochemical composition.

The obtained results demonstrate that *U. intestinalis* has potential for aquaculture incorporation, however, systems need to be setup as to not create conditions driving asexual reproduction.

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### Abstract

Sea urchin gonads, commonly known as “uni” (the Japanese word for “sea urchin”), are a gourmet food that has, for the past couple of years, been hailed as the “Portuguese Caviar”. Sea urchins are traditionally consumed both in Asia and in Mediterranean Europe, but lately their popularity has been expanding all over the world as a novel delicacy. Unfortunately, this newfound interest has the downside of driving the overexploitation of natural resources. *Paracentrotus lividus*, the most common species in the Portuguese coast, is the most commercially exploited echinoid in Europe, and their populations reached alarming levels due to illegal and indiscriminate catches. While few commercial sea urchin farms have been implemented, the technology for their production still needs developing to achieve an economically and ecologically viable industry. The goal in a sea urchin farm is not only maximizing production yield but mostly increasing quality, which drives value. However, with the overexploitation of natural populations, individuals with commercial size are becoming ever scarcer. As such, aquaculture units may have to rear the individuals to market size. As sea urchins may take a long time to grow, a cheap feed source is of the utmost importance to make their production viable. With this in mind, we studied the effect of three different food sources on sea urchin growth, using agar as a matrix: a green seaweed, (*Ulva* sp.), a brown seaweed, (*Saccorhiza polyschides*); and cereals and fish meal, mimicking a practical feed provided to aquaculture fish. Experiments were conducted indoors with no illumination and at a controlled temperature of  $18 \pm 1$  °C.

Growth rates (initial size  $3.6 \pm 0.2$  cm), in diameter and weight (close to 0.1 cm/month and 0.45g/month, respectively), were high and did not significantly differ with feed provided. Weight increase is usually associated with gonad development, but, as individuals were below commercial size, sacrificing the animals to observe their gonads would have been vain.

Despite the optimistic results, sea urchin growth is highly seasonal and influenced by environmental conditions. As such these results need to be validated year round and in a system without controlled conditions. Also, while agar has the advantage of having a high water stability and facilitating the inclusion of other ingredients, it is an expensive resource that needs to be substituted by a more economically viable alternative.

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### Abstract

Larval and early juvenile production phases of whiteleg shrimp *Penaeus vannamei* are the most critical in aquaculture. Relatively low survival rates and sub-optimal quality are associated to inadequate feed and feeding practices and incidence of cannibalism, which affects productivity and profitability of farming enterprises. In this context, two growth trials were conducted with postlarvae and juveniles of *P. vannamei*, respectively, in a recirculation aquaculture system at RiaSearch facilities, to compare the survival and growth of individuals fed with different types of feed and feeding frequencies.

In the first trial, two feeding protocols were tested. One consisted of a co-feeding regime combining a commercial inert microdiet and *Artemia nauplii* during the first eight days, followed by the inert microdiet alone until the end of the trial; the other regime consisted in feeding the commercial inert microdiet alone during the entire trial. Shrimp survival was not significantly different among the feeding protocols, but a higher growth was observed for co-fed postlarvae, whose final body weight and daily growth index were, respectively, 96% and 67% higher than that of non-co-fed postlarvae. This represents a considerable improvement of *P. vannamei* postlarvae growth.

In the second trial, shrimp juveniles were fed using three different feeding frequencies – 4, 6 and 8 times per day. Survival was not affected by feeding frequency, but the juveniles given 8 meals per day showed the highest growth performance, with a final body weight 53% and 11% higher than those fed 4 and 6 times a day, respectively. Additionally, feed efficiency in shrimp fed 6 and 8 times per day was, respectively, 1.4 and 1.6-fold higher than that of shrimp fed 4 times a day. This indicates that feeding 8 times per day remarkably improves growth performance and feed utilization efficiency of *P. vannamei* juveniles.

Results of the present studies demonstrate that using co-feeding regimes with *Artemia nauplii* and inert diets, during early developmental stages, and providing 8 meals per day are the best feed and feeding management to improve survival and growth of *Penaeus vannamei* at RiaSearch, thus enhancing profitability.

**Keywords:** Feeding protocol; Whiteleg shrimp; Cannibalism; Growth; Co-feeding; Feeding frequency; *Penaeus vannamei*